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Tree canopy biodiversity in the Great Smoky Mountains National Park: ecological and developmental observations of a new myxomycete species of *Diachea*

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Abstract: A survey and inventory of tree canopy biodiversity for cryptogams (myxomycetes, macrofungi, mosses, liverworts, lichens and ferns) in the Great Smoky Mountains National Park resulted in the discovery of an undescribed myxomycete species. This taxon is classified in the order Physarales, family Didymiaceae and genus *Diachea*. A combination of morphological characteristics distinguishes *Diachea arboricola* H.W. Keller & M. Skrabal sp. nov. from all other species in the genus: peridium iridescent gold to silvery gray; stalk reddish orange above and whitish below, filled with crystals; capillitial threads stiff, dichotomously branched and arising from the tip of the columella; spore ornamentation uniformly covering the entire spore surface, appearing spiny with light microscopy, with scanning electron microscopy as vertical processes with capitate, clustered, spike-like tips. This type of spore ornamentation has not been found in any other *Diachea* species. *Diachea arboricola* is known only from the tree canopy, ranging in height from roughly 3 to 21 m, on three tree species, *Fraxinus americana, Juniperus virginiana* and *Quercus alba*. Observations of plasmodial growth and fruiting body development are described based on moist chamber cultures. Tree canopy observations in situ suggest that the plasmodium of this species migrates over extensive vertical areas of tree bark. Ecological factors are discussed that include pH of bark substrata. The species description is based on abundant sporangia from 17 different collections. A key to the species of *Diachea* is provided to aid in the identification of this taxon.

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INTRODUCTION

The objectives of this study were: to complete the first comprehensive survey and inventory of tree canopy biodiversity for cryptogams (myxomycetes, macrofungi, mosses, liverworts, lichens and ferns) in the Great Smoky Mountains National Park (GSMNP); to collect these targeted groups of organisms above 3 m on a vertical transect to the treetops; to assemble a multidisciplinary research team of experts who would coordinate the collection, identify and curate this diverse group of organisms; to compare the assemblages of tree canopy targeted organisms with those of ground sites; to search for species new to science in all of the targeted groups of organisms; and to involve undergraduate and graduate students in an adventure phase of tree climbing and sampling from the tree canopy. Details of our tree canopy field studies in the GSMNP were documented in several publications (Counts et al 2000, Henley et al 2000, Skrabal et al 2001, Keller 2002, Keller et al 2002, Keller and Skrabal 2002, Keller and Snell 2002, Snell and Keller 2003, Snell et al 2003). This paper describes a new species of *Diachea* in the tree canopy based on 17 collections, records the ecology of this species in the field, describes its growth and development in moist chamber culture, analyzes bark pH for the tree species *Fraxinus americana* L. (white ash), *Juniperus virginiana* L. (eastern red cedar) and *Quercus alba* L. (white oak) where it was found, and provides a key to species of *Diachea*.

MATERIALS AND METHODS

Study area.—The Great Smoky Mountains National Park of more than 210 566 ha serves as a refuge for one of the richest and most diverse biota in the temperate regions of the world. It also has the largest remaining tracts of old-growth forest in Eastern United States, ca 40 000 ha, and was designated a national park 15 Jun 1934, an International Biosphere Reserve, 26 Oct 1976, and a World Heritage Site, 6 Dec 1983 (FIG. 1). The park is located on the boundary between eastern Tennessee and western North Carolina between 35°28′ and 35°47′N latitude. Elevations range from 263 m to 1994 m. The climate is exceptional for cryptogam

FIGS. 1–4. Cades Cove area Great Smoky Mountains National Park.

FIGS. 5–13. *Diachea arboricola*. 1. Sign at the Townsend, Tennessee, park entrance leading to Cades Cove. 2. Trailhead sign leading to holotype locality. 3. Melissa Skrabal using double-rope climbing method to ascend *Quercus alba* tree No. 88. Sporangia and plasmodial tracks were observed directly on bark. 4. Skrabal collecting bark samples. Note harness and climbing rope enable the climber to use both hands to remove bark samples with a heavy-bladed knife and fill a collection

growth with maritime tropical air bringing year-round moisture, averaging 216 cm annually. Moderate temperatures range from 4 to 23 C at lower elevations (Shanks 1954).

All Taxa Biodiversity Inventory.—A new research initiative called the All Taxa Biodiversity Inventory (ATBI), under the rubric of a nonprofit organization, Discover Life in America, represents a research effort to inventory all life forms in the park. No previous study has included collections of cryptogams from the tree canopy. The high canopy of the old growth forest allowed for collection from a great range of heights, occasionally more than 40 m.

Sampling methods.—A total of 240 trees representing 35 tree species were climbed 19 Jun–6 Jul and 31 Jul–17 Aug 2000 and 9 Jun–28 Jun and 22 Jul–9 Aug 2001. Tree selection often was limited by climbing hazards, such as poison ivy (*Toxicodendron radicans* [L.] Kuntze), dead branches or weak limb structure. Acceptable trees exceeded 40 cm diam and 30 m in total height, which allowed sampling from approximately tree base to treetop. Individual trees were selected from geographically different areas of the park, but due to access and climbing restraints random selection was not practical. Only living trees in healthy condition, without any apparent sign of disease or dead parts, were selected for climbing. Our tree climbing was concentrated along the northern boundary of the park, extending from the Cades Cove area in the western end of the park (FIGS. 1, 2) to Albright Grove in the east. The new species of *Diachea* was collected on three tree species, *Fraxinus americana, Juniperus virginiana* and *Quercus alba,* in the Cades Cove area.

Bark samples were collected using the double-rope climbing technique (Jepson 2000). The climbing details of accessing the tree canopy using the double-rope climbing technique were described elsewhere (Counts et al 2000, Keller 2002, Keller and Skrabal 2002, Snell and Keller 2003). Bark samples were taken at roughly 3 m increments usually from 3 to 30 m as the climber advanced upward (FIG. 3). Bark was scraped or pried from the trunk using a large knife, taking care not to damage the underlying living tissues (FIG. 4). Efforts were made to sample all sides of the trunk. All samples were collected from living parts of the tree and placed in paper bags (ca 1000 cm^3), on which height and tree number were recorded. Trunk diameter at breast height (DBH = 1.5 m) was measured for each tree, and voucher specimens of leaves were gathered for positive identification. Each climber used an elevation line marked off in 0.3 m increments attached to the climbing harness to determine tree height. The total tree height was estimated by the climber. Data from each tree climbed were entered into a database that included a tag number for each tree (FIG. 3), numbered samples at 3 m increments up to 40 m measured with elevation lines, tree diameter at breast height in centimeters, total height of tree in meters, climber's name, observations of in situ specimens on the trunk (FIG. 5), place location description and global positioning system reading, altitude and weather conditions. All student climbers were given tutorials by experts from the multidisciplinary research team that included lecture slide shows, demonstration of specimens, how to key specimens and field identification. This enabled the student climbers to recognize and collect the targeted groups of organisms (Keller 2004).

Tree canopy definition.—Tree canopy is defined as a vertical transect beginning at 3 m and extending to the crown of living trees. Canopy structure is the organization in space and time of the above-ground components of the vegetation (Parker 1995). This more general definition includes bark surfaces of living trees below the first branches in the crown of individual trees. Trees were selected in part because of their size in diameter and in total height, usually those with a minimal height of 30 m. Trees with large-diameter bases with buttress roots and epiphytic mosses and liverworts often support myxomycete plasmodia and fruiting bodies that are more typical ground species (e.g., *Lycogala flavofuscum* [Ehrenb.] Rost). This assemblage of myxomycete ground species at the base of trees occurs below 2 m (Keller and Braun 1999).

Laboratory methods.—Bark samples were examined with a dissecting microscope for myxomycete fruiting bodies before being cultured. Myxomycetes were cultured from tree bark using the moist chamber technique described by Keller and Braun (1999) and Snell and Keller (2003). Moist chambers consisted of large sterile Petri plates (150 × 25 mm) fitted with a sheet of P8-creped filter paper that covered the dish bottom. Bark was placed face up on the paper in a single layer. Sterile distilled water adjusted to pH 7 with potassium hydroxide was poured into the plate around the

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bag. 5. Network of plasmodial tracks on field-collected bark from tree 88 4×. 6. *Juniperus virginiana* (tree 174) next to fence line along Cades Cove Loop Road. Plasmodial tracks and sporangia were observed directly on the bark. Note the open field habitat and pyramidal shape typical of this tree species. Student climber Danny Pacholski's white shirt barely can be seen near the top of the tree. 7. Three separate yellowish phaneroplasmodia migrating over surface of filter paper in moist chamber culture, approximately actual size. 8. Yellow phaneroplasmodium growing on white filter paper in moist chamber culture. Note the feeding, advancing fan and trailing veins that leave plasmodial tracks on the bark surface of tree bark 1.5×. 9. Network of plasmodial veins as part of a living phaneroplasmodium covering the filter paper in moist chamber culture 4×. Note resemblance to plasmodial tracks in FIG. 5. 10. Sporangial primordia in early stages of development, approximately actual size. 11. Immature sporangia in later stages of development after spore cleavage. Note gregarious habit as a single plasmodium gives rise to many sporangia and distinctly colored reddish-orange stalks with whitish base 14×. 12. Single immature sporangium shown in profile. Spores mature and dark brown in mass 50×. 13. Mature sporangium with intact, iridescent peridium with glittering gold, silver and bluish colors 50×.

bark, and the lid was replaced to maintain a closed moist chamber. After 24 h any unabsorbed water was decanted and the pH of each culture was measured using an Orion model 610 flat probe pH meter. Plates were kept moist, without standing water, at room temperature (22–25 C) in indirect natural light. Each plate was examined every 7–10 d, starting 24–48 h after wetting. Moist chamber cultures were kept wet and observed 4 wk.

Collection and identification methods.—Species description follows the terminology of Keller and Braun (1999). Myxomycete fruiting bodies were collected as field specimens or harvested from moist chambers, then preserved in boxes as described by Keller and Braun (1999). Field collections are indicated by (fc), moist chamber cultures by (mc) and total vertical height of the specimen on the tree as (th). Collection numbers from the tree canopy were reported by Kenneth L. Snell as KLS followed by the tree number and by Harold W. Keller as HWK followed by the tree number (Snell et al 2003). All tree canopy collections were entered into the Discover Life in America GSMNP-ATBI database. This database included latitude and longitude (GPS) readings, place locations, county, elevation, collection date and collector. The species description was based on light microscopy (LM) and scanning electron microscopy (SEM). Macroscopic measurements were taken from 100 mature sporangia from field collections and moist chamber cultures. Measurements were based on minimum, maximum and average numbers. Sporangia were examined in water mounts because lactophenol dissolves calcareous structures. Critical-point treated specimens were sputter coated with chromium to an estimated metal thickness of about 4 nm in an Edwards Xenosput 2000 apparatus. Scanning electron micrograph pictures were taken using a Zeiss 982 Gemini field emission instrument with an in lens detector for secondary electrons. Color notations (Kelly 1965, McKnight 1977) in parentheses are included in the species description based on color standard charts. Author abbreviations for species follow Brummitt and Powell (1992).

TAXONOMY

The genus *Diachea* with its glittering gold, bronze or bluish iridescent peridium, along with the color contrast of the calcareous white or colored stalk, imparts a beauty seldom matched by other myxomycetes. The taxonomic history of the genus *Diachea* emphasizes the iridescent peridium and noncalcareous capillitial system similar to *Comatricha* or *Lamproderma,* including the genus in the order Stemonitales and family Stemonitaceae (Martin and Alexopoulos 1969). In contrast, emphasis on morphological characters, such as the calcareous stalk and columella composed of either granules or crystals, places *Diachea* in the order Physarales and in either the family Physaraceae (Lister 1925, Hagelstein 1944) or Didymiaceae (Martin et al 1983, Keller and Braun 1999).

Diachea arboricola H.W. Keller & M. Skrabal, sp. nov. FIGS. 5, 7–22

Sporangia gregaria, saepe stipitate, usque 1.3 mm alta vel sessilia, globosa, 0.4–0.7 mm; peridium membranaceum persistens, iridescens, aurem (84.valde flavum) vel argenteocanum (264. pallide canum), base annulo iridescenti-azureo (179. saturate azureo); dehiscentia in statu maturitatis naturalis, ex apice, irregularis, plerumque residues basalibus; hypothallus indistinctus; stipes arrectus, crystallis calcareis omnino praeditus, usque 0.7 mm altus, base expansus 0.2–0.8 mm latus; columella per fabricam stipi similes, subcylindrica, in extremitate sporangiali in apicem angustiorem contracta, 0.14–0.3l mm alta; capillitium ex filis dichotome ramosis laevibus cavis a peridio discretis ex apice columella orientibus, in luch transmissa atro-violaceis (220. peratro-violaceis), ad apices atque ad affixionem columellae pallidioribus compositum; sporae globosae liberae in totae visae atro-brunneae vel nigrae, in luce transmissa parietibus roseolis 1 μm latis, sub miscroscopio lucido subspinosis, in sectione opularia in tota 11–14.5 μm diam. Phaneroplasmodium saturate flavus, conspicuum, 11 cm attingens.

Sporangia gregarious, not closely crowded, varying from sessile to more often stipitate, 1–1.3 mm in total height, spore case globose, 0.4–(0.55)–0.7 mm; peridium membranous, transparent, smooth, fragile, iridescent, ranging from gold (84. strong yellow) to silvery gray (264. light gray) with a ring of iridescent blue (179. deep blue) at the base, appearing colorless without spores as seen with transmitted light; dehiscence occurring irregularly from apex when mature, usually with basal remnants adhering to the columella and stalk; hypothallus not apparent; stalk erect, calcareous, solid, euhedral rhombohedron crystals throughout, height up to 0.76–(0.5) mm diam at midsection 0.08–0.13 mm, expanded at base 0.2–0.8 mm, enclosed by a membrane 2.5 μm thick, wrinkled, reddish orange (37. moderate reddish orange) to pinkish red (26. strong yellowish pink), whitish at base; columella structurally similar to stalk, more or less cylindric, tapering to a narrower point at apex of spore case, height 0.14–0.31 mm; capillitium consisting of dichotomously branched threads, sometimes with cross connections or with a partial meshwork, smooth, hollow, free of peridium, usually arising as stiff threads from apex of columella, dark violet with transmitted light (220. very deep purple), paler at tips and at attachment to columella; spores globose, free, dark brown to black in mass, violet (218. strong purple) with transmitted light, walls pinkish 1 μm thick, ornamentation appearing spiny with truncate ends with light microscopy, 11–(12.8)–14.5 μm diam in optical section including ornamentation, body of spore 9–13 μm diam, spore surface uniformly covered with vertical processes bearing irregular, capitate, spinous tips as seen with scanning electron mi-

croscopy. Phaneroplasmodium bright yellow, conspicuous, extending up to 11 cm.

HOLOTYPE. UNITED STATES OF AMERICA. TENNESSEE: Blount County, Great Smoky Mountains National Park, at the intersection of School House Gap and Turkey Pen Ridge Trail, GPS, N35.33.17–W083.44.179, altitude 559 m, on bark of living *Quercus alba* tree No. 88 (fc) at 17.4 m height, 1 Aug 2000, legit Melissa Skrabal, *Harold W. Keller 4000-88,* deposited at GB in Göteborg, Sweden. ISOTYPES at BPI; at NY; at herbarium GSMNP. PARATYPES at K; BR.

Specimens examined. All specimens were collected in the Great Smoky Mountains National Park (7 [fc] and 10 [mc] collections). TENNESSEE. BLOUNT COUNTY. Off Cades Cove Loop Road, 1.1 km up Rabbit Creek Trail, N36.35.137–W083.51.700, elevation 672 m, living *Fraxinus americana,* (th) 18 m, (mc), wetted 18 1 Nov, harvested 16 Dec 2001, *KL Snell 1313-247*; Cades Cove Loop Road next to fence, N35.36.415–W083.47.265, elevation 565 km, solitary tree in open field, living *Juniperus virginiana,* (th) 3 m, (mc), wetted 20 Mar 2001, harvested 26 Mar 2001, *HW Keller 4661-174*; (th) 3 m, (fc), on epiphytic moss, 17 Aug 2000, *HW Keller 4671-17*; (th) 3 m, (mc), wetted 20 Mar 2001, harvested 26 Apr 2001, *HW Keller 4673-174*; (th) 3 m, (mc), wetted 20 Jun 2000, harvested 30 Jul 2000, *HW Keller 4674-174*; (th) 6.9 m, (mc), wetted 10 Jun 2001, harvested 20 Jun 2001, *HW Keller 4675-174*; intersection of School House Gap and Turkey Pen Ridge Trails, 1.77 km from Laurel Creek Road, N35.38.137–W083.44.172, (th) 17.4 m, elevation 604 m, living *Quercus alba,* (fc), 1 Aug 2000, *HW Keller 4000-88*; (th) 11.4 m, (fc), 1 Aug 2000, *HW Keller 4001-88*; (th) 18 m, (fc), 1 Aug 2000, *HW Keller 4002-88*; (th) 17.4 m, (fc), 1 Aug 2000, *HW Keller 4003-88*; (th) 18 m, (fc), 1 Aug 2000, *HW Keller 4002-88*; (th) 14.4 m, (fc), 4 Jul 2000, *HW Keller 4013-88*; (th) 20.7 m, (mc), wetted 13 Feb 01, harvested 20 Feb 2001, *HW Keller 4653-88*; (th) 6 m, (mc), wetted 15 Jan 2001, harvested 20 Feb 2001, *HW Keller 4654-88*; (th) 20.7 m, (mc), wetted 13 Feb 2001, harvested 8 Mar 2001, *HW Keller 4655-88*; (th) 14.4 m, (mc), wetted 10 Jun 2001, harvested 30 Jun 2001, *HW Keller 4662-88*; (th) 19.8 m, (mc) wetted 26 Mar 2001, harvested 24 Apr 2001, *HW Keller 4672-88.*

Etymology. Arbor from the Latin, meaning tree, and *cola,* meaning to dwell, refers to this species occurring on the trunk bark of living trees above 3 m.

Distribution. United States of America, Great Smoky Mountains National Park.

Habitat. Canopy of living trees, *Quercus alba, Juniperus virginiana* (FIG. 6), and *Fraxinus americana* on bark surfaces and crevices.

Seasonal occurrence. Jun–Aug in Great Smoky Mountains National Park.

Commentary. The distinguishing characters of *D. arboricola* are the combination of peridial and stalk colors and composition, the stiff capillitial threads arising primarily from the tip of the columella, the spore ornamentation and the microhabitat in the canopy of living trees. *Diachea megalospora* Thind & Manocha, *D. silvaepluvialis* M.L. Farr, *D. verrucospora* Nann-Bremek. & Y. Yamam. and *D. thomasi* Rex. all have colored stalks and variable iridescent peridial colors. In all of these species the capillitum arises throughout the length of the columella. The character of the columella is striking in *D. arboricola* because the stiff capillitial threads often project upward from the tip of the columella when the sporangia are windblown. In addition, there is a free space between the tip of the columella and junction of the columella and stalk (FIGS. 17, 19). *Diachea* species have beautiful iridescent peridia but *D. arboricola* is striking because of its glittering golden to silvery colors and the contrasting pink to reddish orange stalk (FIG. 13). Individual rhombohedron crystals typical of calcite are found in the swollen whitish area at the base, throughout the stalk proper, and also in the columella (FIGS. 15, 16). Farr (1974) noted that, in *D. bulbillosa* (Berk. & Br.) A. Lister, sporangia always had crystalline, calcareous stalks from tropical regions in contrast to granular calcareous stalks from the temperate zones. *Diachea silvaepluvialis* and *D. megalospora* also have crystalline, calcareous stalks. *Diachea thomasii* is known only from the United States of America, from ground sites in Pennsylvania, North Carolina, Tennessee and West Virginia. Collections examined clearly show *D. thomasii* to be distinct from all other *Diachea* species based on the bright orange stalks filled with calcareous granules and spore ornamentation of delicate, minute warts with scattered, small clusters of distinctly darker warts. Lado (2001) synonymized *D. verrucospora* with *D. silvaepluvialis* under his list of accepted names. We compared the types of *D. verrucospora* and *D. silvaepluvialis* using LM and SEM, and the two taxa are identical. The general habit, silvery iridescent peridium and brown to dark orange stalk with crystals, capillitial threads that arise throughout the length of the columella, and spore ornamentation of two distinct types, irregularly covered with spines and also with patchy, circular, distinct areas of delicate warts, differ from the combination of characters exhibited by *D. arboricola.*

RESULTS

Ecological observations.—Field-collected sporangia of *D. arboricola* were found above 3 m. Twenty moist chamber cultures from bark samples collected at 2 m from trees Nos. 88 and 20 moist chamber cultures from bark samples from tree No. 174 did not yield plasmodia or sporangia of *D. arboricola.* Tree 88 and tree 174 were climbed at least three different times and each time the sporangia and plasmodial tracks

were found above 9 m on tree 88 and above 3 m on tree 174. During and after long rainy periods plasmodial tracks commonly are seen along with sclerotia on the bark surface of living trees (Keller and Braun 1999). Plasmodial tracks on tree 88 (FIG. 5) extended from 9 to 24 m and represent traces of the plasmodial veins that leave an imprint on the bark surface. The black outline of excreted matter forms along each side of the vein leaving two black lines with a white space in between that marks the bottom position of the vein (FIG. 5).

Diachea arboricola first was discovered by undergraduate student climber Melissa Skrabal on tree 88 and later by Buck Counts on tree 174. This remarkable discovery of these field-collected specimens and the personal experiences of the student climbers were described in more detail in previous publications (Counts et al 2000, Keller and Skrabal 2002, Keller et al 2002, Keller 2004). Plasmodial tracks on tree 88 indicated that this species had colonized the bark surface vertically for approximately 15 m (FIG. 5). In more than 30 years experience of collecting corticolous myxomycetes from the bark of living trees, this *Diachea* plasmodium covered the most extensive area ever observed (Keller, pers obs). Tree 174 was about 12 m in total height (FIG. 6) with plasmodial tracks and sporangia developing from 3 to 9 m. *Badhamia affinis* Rost. also develops plasmodial tracks and sporangia covering extensive areas on living trees, most frequently on *Juniperus virginiana* (Keller and Braun 1999). None of the nine species of *Diachea* is known from the bark of living trees. *Diachea* species are found on ground habitats such as leaf litter, mixed litter of decomposing twigs, wood fragments, leaves and sometimes on the stems of herbaceous plants. *Diachea leucopodia* (Bull.) Rost. develops an extensive white phaneroplasmodium that sometimes migrates over great distances to finally sporulate several meters above ground on living stems and leaves of the common stinging nettle, *Urtica dioica* L. (Keller and Braun 1999). *Diachea arboricola* apparently develops a yellow plasmodial stage that is capable of migrating over great distances but is confined to the upper levels of living trees (FIGS. 5, 7–9). There was no evidence that plasmodia migrated from ground sites up the trunk of living trees, although this does occur in other species such as *Lycogala flavofuscum* (Ehrenb.) Rost., *Physarum didermoides* (Pers.) Rost., and *Stemonitis flavogenita* Jahn (Keller and Braun 1999). Keller and Skrabal (2002) referred to the new species of *Diachea* as an obligate tree canopy species. This designation as an ''obligate canopy species'' probably is premature at this time because, in the case of *J. virginiana,* fruiting bodies of *D. arboricola* were only 3 m above ground. However, *D. arboricola* is another example of a growing list of corticolous myxomycetes that are found only on the bark of living trees and vines and never on ground sites.

Mature sporangia collected directly from the bark surface of tree 88 often were sessile and sometimes aberrant, lacking iridescence from premature drying. The plasmodium apparently migrates to drier areas of the bark either on the surface or in crevices or fissures, sporulating under adverse conditions causing aberrant development. In moist chamber cultures most sporangia were stalked, reaching maximum height apparently due to the more optimal moist conditions during the sporulation period. Other trees in the vicinity of tree 88 were sampled (e.g., *Quercus alba* tree 107, which was within 10 m, but *Diachea arboricola* was never found based on 12 moist chamber cultures at six different heights).

Mean pH is a major factor delimiting the assemblage of myxomycete species on the bark of living trees (Snell and Keller 2003). *Pinus strobus* L. had a mean pH of 3.8 and a distinct group of myxomycete species different from those associated with *Fraxinus americana,* with a mean pH of 6.7 (Snell and Keller 2003). *Diachea arboricola* occurred on: *J. virginiana* (tree 174), with a mean pH of 7.38, SD± = 0.252, N = 85; a *Q. alba* (tree 88), with a mean pH of 6.82, SD± = 0.564, N = 12; and *F. americana* (tree 247), with a mean pH of 6.67, SD± = 0.357, N = 14. *Juniperus virginiana* is a conifer with a slightly basic pH that had an assemblage of myxomycete species similar to *F. americana* but distinctly different from *P. strobus*. The taxa associated with *D. arboricola* on at least two of the three tree species were: *Badhamia rugulosa* T.E. Brooks & H.W. Keller, *Cribraria violacea* Rex, *Diderma chondrioderma* (de Bary & Rostaf.) G. Lister, *Licea kleistobolus* G.W. Martin, *L. pedicellata* (H.C. Gilbert) H.C. Gilbert, *Macbrideola cornea* (G. Lister & Cran) Alexop., *M. decapillata* H.C. Gilbert, *M. scintillans* H.C. Gilbert, *Perichaena chrysosperma* (Curr.) Lister, *P. depressa* Libert, *P. minor* (G. Lister) Hagelst. var. *pardina, Physarum crateriforme* Petch, and *Trabrooksia applanata* H.W. Keller. It is interesting to note that *J. virginiana* is at the basic end of the pH spectrum, has fibrous bark which readily absorbs water and has an assemblage of species similar to *Fraxinus americana,* also with a near neutral pH. It appears that *D. arboricola* has an optimal pH near 7.0 and occurs with tree species that have bark near this pH.

Developmental observations.—Sclerotized plasmodia were not observed on the upper or lower bark surfaces or in furrows on bark before wetting in moist chambers. Plasmodia when first observed already

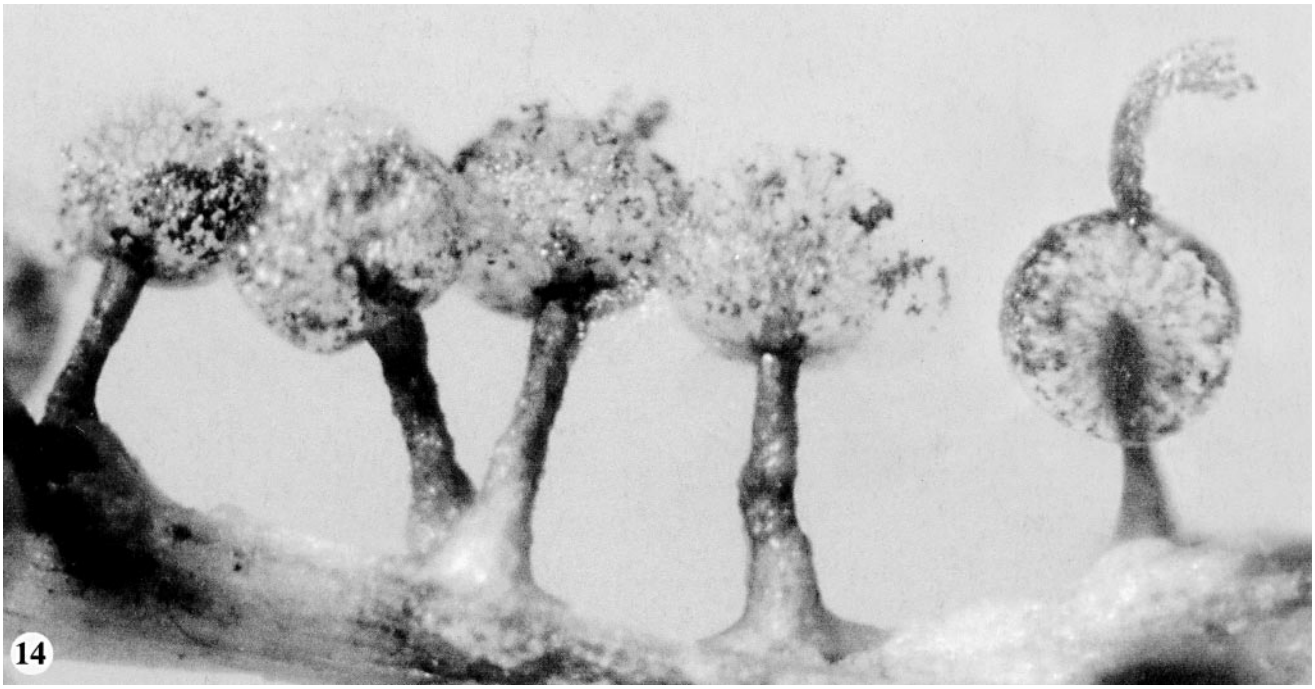
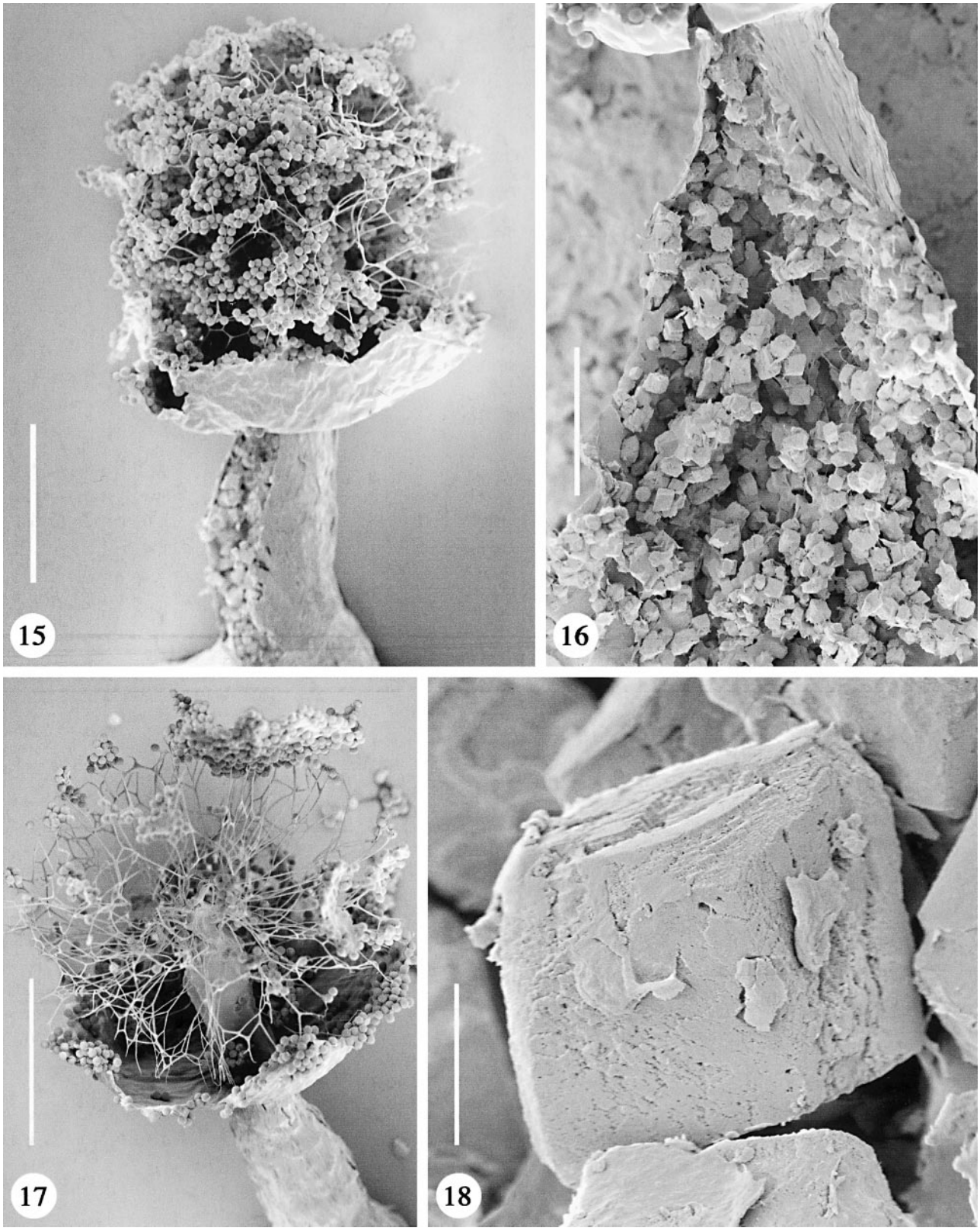


FIG. 14. *Diachea arboricola* (LM). Group of sporangia showing natural dehiscence of delicate, fragile, transparent peridium at surface, bicolored stalks, darker above and white at base, and columella in optical section (far right) 46×.

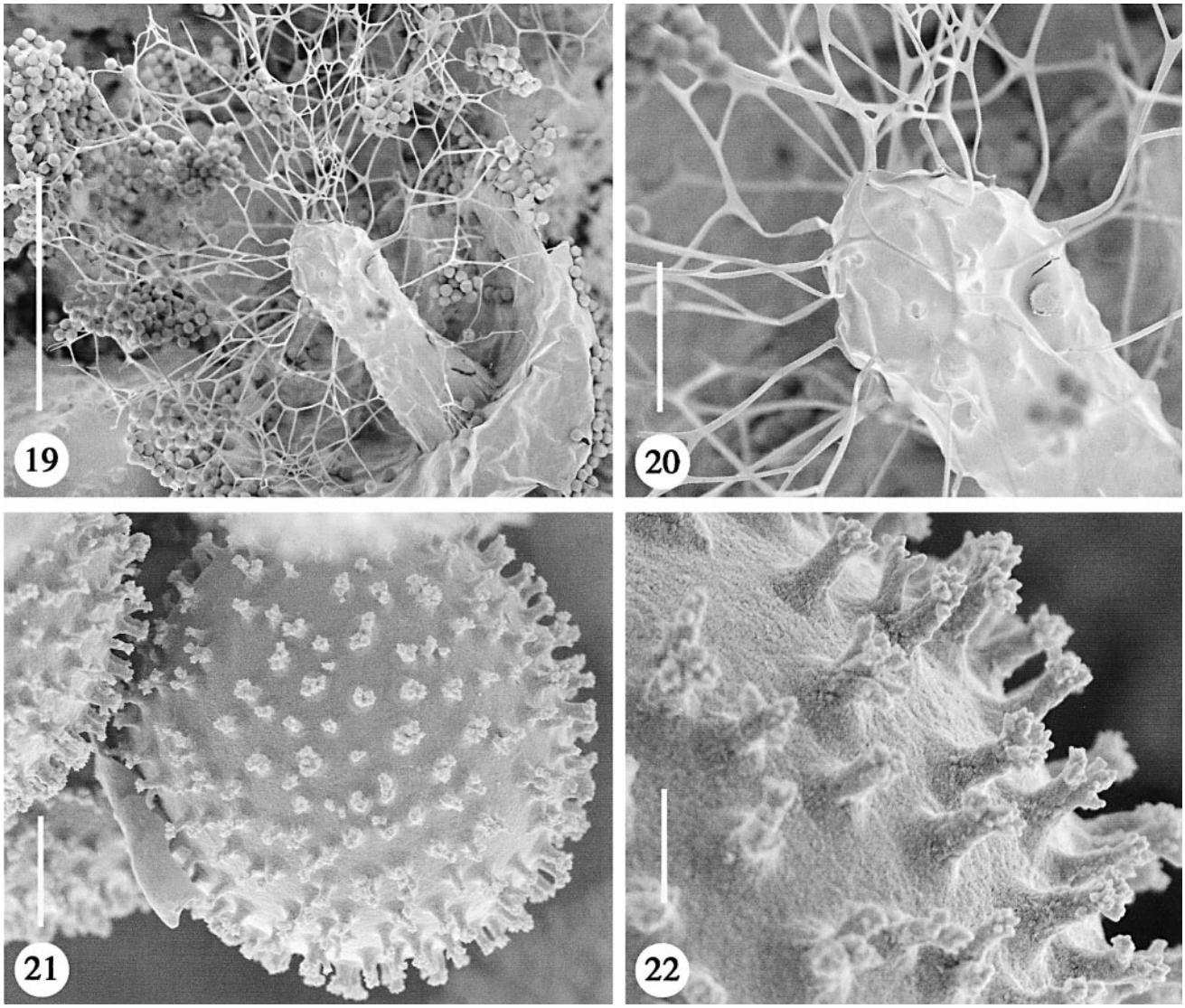
were large, several centimeters across (8 × 5 cm) and migrating over the bark surface or on filter paper with an advancing fan about 4.5 cm across (FIG. 8). There was usually one large plasmodium that spread over the upper and lower bark surfaces, often covering epiphytic mosses and filter paper in the bottom of the Petri dish (FIGS. 7–9).

Plasmodia in culture simulated field conditions where plasmodia migrated over extensive areas. Some plasmodia sclerotized into hard, yellow flattened masses that revived with rewetting; still others developed aberrant prematurely dried sporangia with agglutinated spore masses surrounded with a peridium lacking iridescence. Some plasmodia first were observed 3 d after wetting, and mature sporangia were harvested 6 d later; other cultures took up to 30 d before plasmodia were observed and sporangia were harvested. Sporangia sporulated on upper and lower bark surfaces, on the moss phyllidia, on the filter paper and on the sides of the plastic Petri dishes (FIG. 10). The bright yellow plasmodium, a type called a phaneroplasmodium, is typical of the Physarales. At maturity this plasmodium exhibited polarity and directional movement, terminating in an advancing, fan-shaped feeding edge with a trailing network of veins (FIGS. 8, 9). The plasmodium had a raised, three-dimensional aspect with definite margins. Each phaneroplasmodium produced hundreds of closely spaced sporangia in the area of the advancing fan and along the veins (FIG. 11).

Plasmodia began to sporulate in the morning from 6 to 10 AM (FIG. 10). At this time sporangial primordia were observed undergoing subhypothallic development (Alexopoulos 1973), myxogastroid (Ross 1973) or nonstemonitoid (Mims 1973) (FIG. 10). This type of sporophore development occurs when the plasmodial protoplasm becomes concentrated into hemispherical mounds, with the hypothallus forming on the surface of the plasmodium from the outer slime sheath. These mounds, destined to become the sporophores, increase in size as protoplasm flows from connecting vein-like strands that finally collapse, separating the mounds from one another. The fluid protoplasm migrates from underneath the hypothallus, pushing upward so that each mound eventually elongates into a columnar structure. These columnar structures develop uneven resistance, so that the apex balloons, forming the incipient spore case, while the lower portion forms the incipient stalk. Contraction of the stalk forces the expanding spore case upward. Development proceeds so that the hypothallus, stalk and sporangial wall form from, and are continuous with, the upper external surface of the plasmodium. This pattern of sporophore development supports the inclusion of *Diachea* in the



FIGS. 15–18. *Diachea arboricola* SEMs of sporangia. 15. Whole sporangium, showing spores intermingled with capillitium. Peridium flakes off, leaving basal remnants or collar. Stalk split open showing calcium carbonate crystals. 16. Fractured stalk showing individual crystals surrounded by a persistent membrane. 17. Windblown sporangium showing top view of capillitial system arising from apex of columella. 18. Individual calcium carbonate rhombohedron crystal. Scale bars: 15, 17 = 200 μm, 16 = 100 μm, 18 = 5 μm.



FIGS. 19–22. *Diachea arboricola.* 19. Profile of capillitium arising from apex of columella showing pattern of branching. 20. High magnification showing attachment of capillitial threads to apex of columella. 21. Uniform pattern of spore ornamentation. 22. Spore ornamentation, showing vertical processes in profile with spike-like projections at tips. Scale bars: 19 = 200 μm, 20 = 50 μm, 21 = 5 μm, 22 = 1 μm.

Physarales and a noncalcareous capillitial system confirms its inclusion in the Didymiaceae.

A series of color changes occurred from bright yellow to reddish brown to chocolate brown indicating that spore cleavage and maturation was taking place (FIGS. 10–12). In approximately 24 h final sporulation was completed (FIG. 12). Final formation of the mature sporangium with iridescent peridium took up to 72 h (FIG. 13). Gradual drying resulted in perfectly formed sporangia with powdery spores (FIGS. 15, 17). The membranous, fragile peridium broke open at the apex due to surface tension from drying (FIG. 14). Irregular peridial fragments fell away, leaving remnants adhering to the stalk as a persistent collar (FIGS. 15, 17). The stalk is filled with calcium carbonate crystals with euhedral rhombohedron shapes (FIGS. 16, 18). These individual crystals probably are calcite because this shape is typical of carbonate minerals. In other taxa, such as *Clastoderma,* the stalk is stuffed with excreted matter, including fungal spores, algae or bacteria, which are deposited from the plasmodial protoplasm (Keller, pers obs). Stalks of *D. arboricola* lack organic matter and consist only of individual crystals (FIGS. 16, 18).

Additional collections of *D. arboricola* from other tree species are needed to determine whether this species is restricted to the upper tree canopy. *Diachea arboricola* is the first myxomycete species new to science where the type locality is located within the GSMNP.

KEY TO THE SPECIES OF *DIACHEA*

Species keyed are *D. arboricola, D. bulbillosa, D. caespitosa* (Sturgis) A & G. Lister, *D. leucopodia, D. megalospora, D. radiata* G. Lister, *D. silvaepluvialis, D. splendens* Peck, *D. subsessilis* Peck and *D. thomasii. Diachea caespitosa* can be difficult to identify because the stalk and columella are often noncalcareous.

1. Spore ornamentation completely spiny-reticulate with 6–10 meshes per hemisphere ..... *D. subsessilis*
1. Spore ornamentation minutely roughened, warted, spiny, or with irregular ridges, not reticulate 2
   2. Sporangia caespitose or crowded, usually sessile ..... *D. caespitosa*
   2. Sporangia gregarious, or closely spaced, usually stalked 3
3. Stalk and columella distinctly colored either orange, pinkish orange, deep yellow or brown 4
3. Stalk and columella white 7
   4. Spore ornamentation spinose with blunt tips uniformly covering entire surface; capillitium arising primarily from tip of columella; on bark of living trees ..... *D. arboricola*
   4. Spore ornamentation uneven with clusters or patches of larger, darker warts, spines or irregular ridges; capillitium arising from throughout the length of columella; on ground sites 5
5. Spore ornamentation with patches of spinose and densely warted areas; stalks brown with slight orange tinge ..... *D. silvaepluvialis*
5. Spore ornamentation with clusters of prominent darker warts; stalks orange 6
   6. Stalks bright orange ..... *D. thomasii*
   6. Stalks light orange with violaceous tints ..... *D. megalospora*
7. Sporangia more or less cylindrical ..... *D. leucopodia*
7. Sporangia globose or nearly so 8
   8. Spore ornamentation coarse, conspicuous, consisting of dark protuberances with irregular ridges ..... *D. splendens*
   8. Spore ornamentation consisting of delicate warts or spines 9
9. Stalk length at least ½ of total height ..... *D. bulbillosa*
9. Stalk length less than ½ of total height ..... *D. radiata*

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LITERATURE CITED

Alexopoulos CJ. 1973. Myxomycetes. In: Ainsworth CG, Sparrow FK, Sussman AS, eds. The fungi: an advanced treatise. Vol. IVB. New York: Academic Press. p 39–61.

Brummitt RK, Powell CE, eds. 1992. Authors of plant names. Royal Botanic Gardens, Kew, England. 732 p.

Counts J, Henley L, Skrabal M, Snell KL. 2000. Tree canopy biodiversity in the Great Smoky Mountains National Park. Inoculum 51(6):1–5.

Farr ML. 1974. Some new myxomycete records for the Neotropics and some taxonomic problems in the Myxomycetes. Proc. Iowa Acad. 81:37–40.

Hagelstein R. 1944. The Mycetozoa of North America. Published by the author. Mineola, New York. 306 p.

Henley L, Skrabal M, Snell KL, Counts J, Keller HW. 2000. Life in the treetops at Great Smoky Mountains National Park. What's Up, The Newsletter of the International Canopy Network 7(1):6–7.

Jepson J. 2000. The tree climber's companion, a reference and training manual for professional tree climbers. Longville, Minnesota: Beaver Tree Publishing. 104 p.

Keller HW. 2002. Tree canopy and corticolous myxomycetes. In: Rammeloo J, Bogaerts A, eds. Fourth International Congress on Systematics and Ecology of Myxomycetes, Abstracts. Scripta Botanica Belgica 22:48 National Botanic Garden of Belgium, Meise, Belgium. 109 p.

———. 2004. Tree canopy biodiversity: student research experiences in Great Smoky Mountains National Park. Systematics & Geography of Plants 74:47–65.

———, Braun KL. 1999. Myxomycetes of Ohio: their systematics, biology, and use in teaching. Columbus, Ohio: Ohio Biological Survey Bulletin New Series. 13. No. 2. 182 p.

———, Skrabal M. 2002. Discovery of a new obligate tree canopy myxomycete in the Great Smoky Mountains National Park. Inoculum 53(2):1–4.

———, Skrabal M, Eliasson UH, Gaither T. 2002. A new tree canopy myxomycete in the Great Smoky Mountains National Park. In: Rammeloo J, Bogaerts A, eds. Fourth International Congress on Systematics & Ecology of Myxomycetes, Abstracts. Scripta Botanica Belgica 22:49–50. National Botanic Garden of Belgium. Meise, Belgium. 109 p.

———, Snell KL. 2002. Feeding activities of slugs on Myxomycetes and macrofungi. Mycologia 94:757–760.

Kelly KL. 1965. ISCC-NBS Color-Name Charts Illustrated with Centroid Colors. Standard Sample No. 2106 Suppl. to National Bureau of Standards Circular 553. U.S. Government Printing Office, Washington, D.C.

Lado C. 2001. Nomenmyx. A nomenclatural taxabase of the Myxomycetes. Cuad Trab Fl Micol Iber 16:1–224.

Lister A. 1925. A Monograph of the Mycetozoa being a descriptive catalogue of the species in the herbarium of the British Museum. Ed. 3. Revised by G. Lister. London, British Museum of Natural History. 296 p.

Martin GW, Alexopoulos CJ. 1969. The Myxomycetes. Iowa City: University of Iowa Press. 561 p.

———, ———, Farr ML. 1983. The genera of Myxomycetes. Iowa City: University of Iowa Press. 102 p.

McKnight KH. 1977. A note on the ISCC-NBS Centroid Charts. McIlvainea 3:4–11.

Mims CW. 1973. A light and electron microscope study of sporulation in the myxomycete *Stemonitis virginiensis*. Protoplasma 77:35–54.

Parker GG. 1995. Structure and microclimate of forest canopies. In: Lowman MD, Nadkarni NM, eds. Forest canopies. San Diego, California: Academic Press. p 73–106.

Ross IK. 1973. The Stemonitomycetidae, a new subclass of myxomycetes. Mycologia 65:477–485.

Shanks RE. 1954. Climates of the Great Smoky Mountains. Ecology 35:354–361.

Skrabal M, Snell KL, Henley L, Counts J, Keller HW. 2001. Fungi in the canopy—Great Smokies Survey. The Mycophile, Newsletter of the North American Mycological Association 42(1):6, 7–13.

Snell KL, Keller HW. 2003. Vertical distribution and assemblages of corticolous myxomycetes on five tree species in the Great Smoky Mountains National Park. Mycologia 95:565–576.

———, ———, Eliasson UH. 2003. Tree canopy myxomycetes and new records from ground sites in the Great Smoky Mountains National Park. Castanea 68:97–108.